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Effects of Pretreatment With Luteinizing Hormone Releasing Hormone (LHRH) on Behaviors Induced by Apomorphine in Rats

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MORA, S. AND G. DIAZ-VELIZ. *Effects of pretreatment with luteinizing hormone releasing hormone (LHRH) on behaviors induced by apomorphine in rats.* PHARMACOL BIOCHEM BEHAV 31(2) 291–296, 1988.—The influence of LHRH on the behavioral effects induced by apomorphine (APO) was studied in male rats. Several doses of apomorphine (31.25, 62.5, 125, 250 and 500 μg/kg) were administered subcutaneously (SC) after LHRH 100 μg/kg or solvent. Low doses of apomorphine induced hypomotility and impaired acquisition of a conditioned avoidance response (CAR). High doses produced hypermotility, stereotyped sniffing and a short lasting increase, followed by a decrease in the acquisition of CARs. Pretreatment with LHRH potentiated the hypomotility induced by low doses of apomorphine (62.5 and 125 μg/kg) and the hypermotility, stereotyped sniffing and the enhancement in acquisition of CARs produced by higher doses of apomorphine (250 and 500 μg/kg). These findings suggest that LHRH could indirectly regulate dopamine activity through an increase in sensitivity of dopamine receptors (pre- and postsynaptic), which mediate the behavioral effects of APO. It is postulated that this hypersensitivity of DA receptors could be the consequence of an inhibition of presynaptic dopaminergic transmission, induced by LHRH.

LHRH Apomorphine Dopamine Conditioned avoidance Behavior Spontaneous motor activity Stereotypy Sniffing

THE decapeptide luteinizing hormone releasing hormone (LHRH) has demonstrated to induce behavioral effects which are probably not related to its stimulatory action of the secretion of pituitary hormones. Small doses of synthetic LHRH, whether administered systemically or infused into the brain, can potentiate sexual behavior patterns in the rat. It has been suggested that LHRH could modulate sexual performance, either directly or indirectly, through a catecholamine system (17). Other pharmacological effects of LHRH on behavior have been described: reduction in the barbiturate induced sleeping time (4), potentiation of the stimulant properties of L-DOPA and 5-HTP in pargyline-pretreated mice (19) and inhibition of the extinction of pole-jumping avoidance responses (7).

We have demonstrated that LHRH can alter the acquisition and retention of avoidance conditioned responses and antagonize the stimulatory actions of amphetamine. In fact, pretraining administration of LHRH impairs the acquisition of an active avoidance conditioned response (10,12) and improves the retention of this behavior when it is injected immediately after training (13). In addition, LHRH has been shown to increase and impair the retention of a passive avoidance conditioned task, according to the intensity of the footshock applied during training (13). Pretreatment with LHRH blocked the stimulatory action of amphetamine in acquisition of conditioned avoidance responses (CARs), spontaneous motor activity and rearing behavior (11). Moreover, L-DOPA antagonized the impairment in acquisition of CARs and it was also able to counteract the antagonism between LHRH and amphetamine in acquisition of CARs and spontaneous motility (14). These findings led us to the suggestion that LHRH could exert its behavioral effect through an inhibitory action upon presynaptic dopaminergic mechanisms, since the integrity of the dopamine (DA) system is important for the stimulant effects of amphetamine.

The present work was carried out to further test the idea of an interaction between LHRH and DA systems. In order to verify that the DA receptors were still functionally intact

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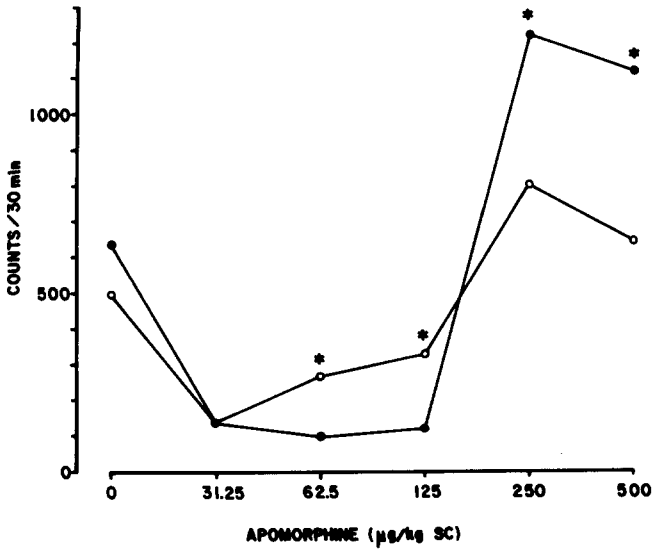

FIG. 1. Effects of the pretreatment with LHRH 100 µg/kg SC (filled circles) or solvent (open circles) on the spontaneous motor activity (SMA) changes induced by increased doses of apomorphine (APO). Each point of the curve represents the median of the total SMA counts in 30 min. Statistical evaluation was made by means of the Kruskal-Wallis one-way ANOVA followed by the Mann-Whitney U-test (*$p<0.05$ and **$p<0.005$). The number of animals in each group was 10.

after LHRH, we studied the influence of LHRH on the apomorphine-induced effects upon motor activity and active avoidance conditioning. Apomorphine is considered as a powerful dopamine agonist which can induce biphasic behavioral effects in rats. In fact, whereas the administration of small doses of apomorphine produces hypomotility, high doses result in stereotypy and locomotor stimulation. These results have been explained by the concept that the behavioral effect of low doses of apomorphine are a consequence of the activation of dopamine autoreceptors and the behavioral effects of high doses are compatible with stimulation of postsynaptic dopamine receptors (20). Briefly, our study demonstrates that LHRH modifies the behavioral effects induced by apomorphine. In general, the effects of apomorphine were potentiated after LHRH treatment.

METHOD

Animals

A total of 210 male Sprague-Dawley rats weighing 180–200 g were used for the whole investigation. They were housed in groups of six under controlled conditions of light (8:00 to 20:00 hr) and temperature (23±2°C) and were allowed free access to standard laboratory diet and tap water. All animals were used only once and were always tested between 10:00 and 16:00 in a sound-attenuated and temperature-regulated room.

Drugs

Drugs were administered subcutaneously (SC) in the dorsal part of the neck. Luteinizing hormone releasing hormone (LHRH, Sigma Chemical Co.) was dissolved in 2% benzyl alcohol and administered at a dose of 100 µg/kg. Apomorphine hydrochloride was dissolved in saline mixed with 2% sodium bisulphite and administered 120 min after LHRH at doses of 31.25, 62.5, 125, 250 and 500 µg/kg. In all cases the doses to be injected were in a volume of 0.1 ml/100 g of body weight. Control animals received the respective solvent.

Spontaneous Motor Activity

Apparatus. Motor activity was measured by using an activity platform (Lafayette Instrument Co.) connected to an electromechanical counter. In order to avoid the influence of disturbing noises the platform was placed into a sound-proof chamber and the observation were made through a closed TV-circuit.

Procedure. Immediately after apomorphine injection each animal was placed on the platform and the spontaneous motor activity was recorded every 5 min during a period of 30 min. Simultaneously the following behavioral elements were also scored: rearings, head shakings, time spent in grooming and sniffing. Stereotyped sniffing was scored according to the following scheme: 0=absent or not different from controls; 1=present intermittently; 2=present continuously with locomotor activity; and 3=present continuously in absence of locomotor activity. The rat's behavior was continuously recorded on videotape from the moment it was placed in the chamber until the end of the session. The tapes were analysed by two trained investigators in order to minimize experimenter bias.

Active Avoidance Conditioning

Apparatus. The conditioning experiments were carried out with a two-way shuttle box (Lafayette Instrument Co.) composed of two stainless steel modular testing units. Each modular chamber was equipped with an 18-bar insulated shock grid floor, two 28 V DC lights and two tone generators (Mallory Sonalert 2800 Hz). Electric shock was provided to the grid floor by a Master Shock Supply (Lafayette Instrument Co.).

Procedure. Immediately after the apomorphine treatment each animal was placed in the shuttle box and, after an habituation period of 5 min, it was trained over 60 trials. Each trial consisted of the presentation of a tone which after 5 sec was overlapped with a 0.25 mA footshock until the animal escaped to the opposite chamber. A conditioned avoidance response (CAR) was defined as a crossing within 5 sec. Intertone interval was 30 sec.

Data Analysis and Statistics

The results were analyzed using nonparametric statistical methods as described by Siegel (22). Kruskal-Wallis one-way analysis of variance followed by a Mann-Whitney U-test was applied to evaluate statistical differences between independent groups. In all cases statistical differences were considered significant when p was equal to or less than 0.05.

RESULTS

Spontaneous Motor Activity

Figure 1 shows the 30-min motility counts obtained by giving various doses of apomorphine after LHRH treatment. Kruskal-Wallis ANOVA from all groups indicated significant group differences, $H(11)=143.03$, $p<0.005$. Increased dosages of apomorphine induced a dual effect in the activity

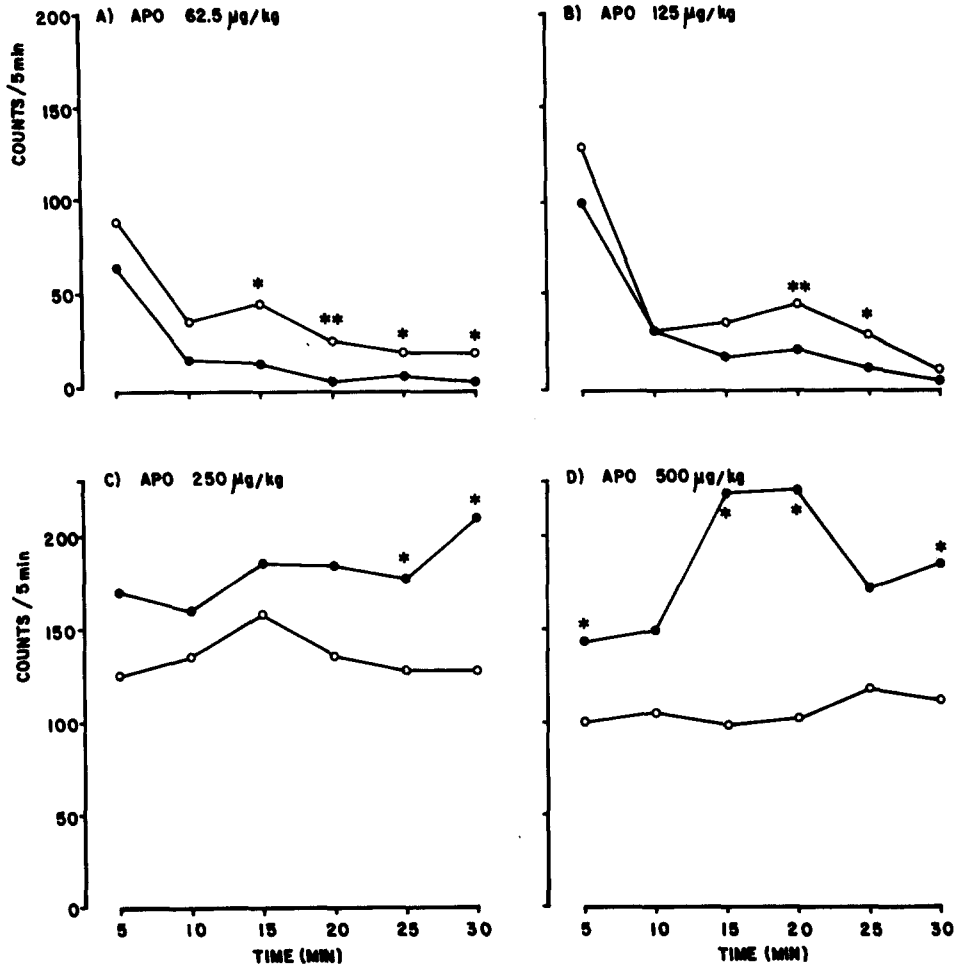

FIG. 2. Time course of the interaction between LHRH and four doses of apomorphine (APO) on spontaneous motor activity (SMA). In each group, animals were pretreated with LHRH 100 µg/kg (filled circles) or solvent (open circles). Each point of the curve represents the median of SMA counts by 5-min intervals after APO injection. Comparisons were made by using Mann-Whitney U-test (*$p<0.05$ and **$p<0.005$). The number of animals in each group was 10.

of rats. Mann-Whitney U-tests indicate that low dosages (31.25 and 62.5 µg/kg) significantly decreased SMA and that the two higher doses produced hyperactivity. LHRH by itself was not able to modify spontaneous motility in the controls, but potentiated both the hypomotility and the hypermotility induced by apomorphine.

Figure 2 shows the time course of the interaction between LHRH and each dose of apomorphine on SMA. Figure 2A shows the effect of LHRH on hypomotility induced by apomorphine 62.5 µg/kg. LHRH potentiated the effect from 10 min through 30 min after the apomorphine treatment compared with solvent. In Fig. 2B, the hypomotility induced by 125 µg/kg of apomorphine was potentiated by LHRH at 20 and 25 min after APO. Figure 2C illustrated the influence of LHRH on the hypermotility induced by apomorphine 250 µg/kg. LHRH significantly potentiated this effect at 20 and 25 min after apomorphine. In Fig. 2D the hypermotility induced by 500 µg/kg of apomorphine was markedly potentiated at 5, 15, 20 and 30 min after apomorphine injection.

Stereotypy

Figure 3 illustrates the influence of LHRH on the stereotyped sniffing induced by the higher doses of apomorphine (250 and 500 µg/kg). Kruskal-Wallis ANOVA from these groups indicated significant group differences, $H(3)=16.69$, $p<0.005$. LHRH significantly potentiated the stereotyped sniffing produced by apomorphine 500 µg/kg. Figure 4 shows that LHRH enhanced the stereotypy at 5, 25 and 30 min after the apomorphine treatment compared with the solvent.

Other behavioral responses, such as head shaking, rearing and grooming were reduced after increased dosage of apomorphine; nevertheless, these effects were not significantly modified by LHRH pretreatment, in our experimental conditions.

Conditioned Avoidance Responses (CARs)

The effects of the interaction between LHRH and the

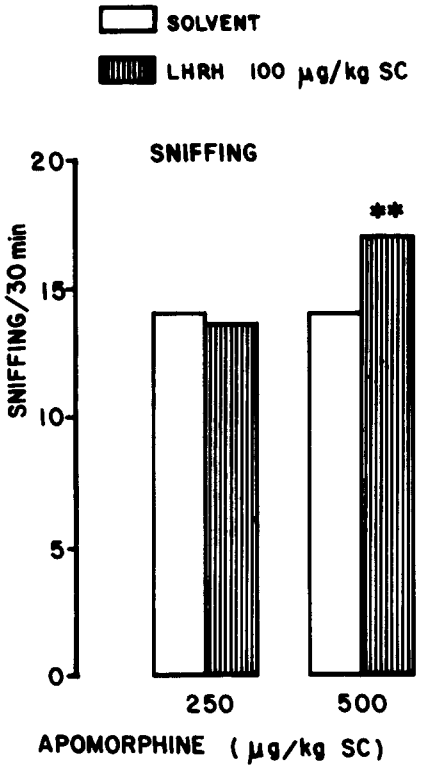

FIG. 3. Influence of the pretreatment with LHRH 100 µg/kg or solvent on the stereotyped sniffing induced by apomorphine. The bars represent the medians of the total scores of stereotypy during the measured period. Statistical evaluation was made by means of the Kruskal-Wallis one-way ANOVA followed by the Mann-Whitney U-test. (**$p<0.005$). The number of animals in each group was 10.

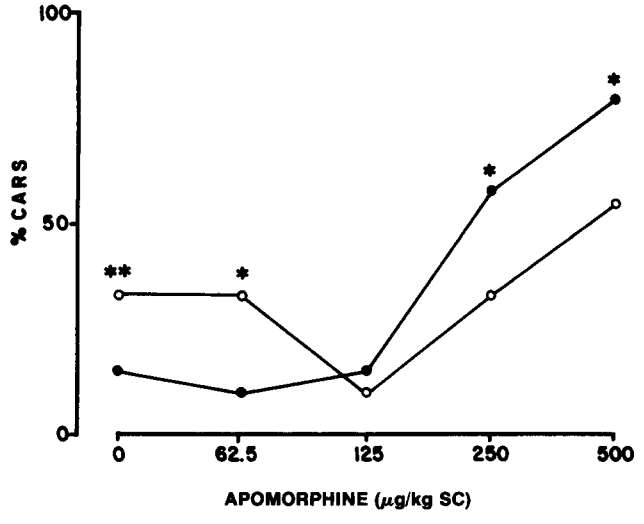

FIG. 5. Effects of the interaction between LHRH 100 µg/kg SC (filled circles) or solvent (open circles) and increasing doses of apomorphine (APO) on the acquisition of conditioned avoidance responses (CARs). Each point of the curve represents the median of the percentages of CARs out of 60 trials. Comparisons were made by using Kruskal-Wallis one-way ANOVA followed by Mann-Whitney U-test (*$p<0.05$ and **$p<0.005$). Number of animals in each group was 9.

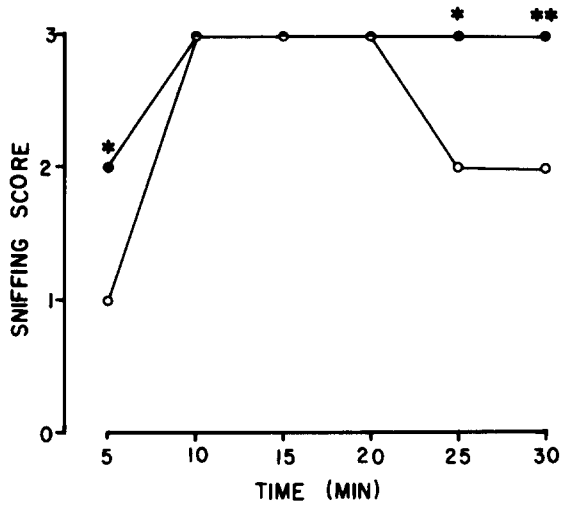

FIG. 4. Time course of the potentiating effects of LHRH 100 µg/kg SC on the stereotypy sniffing induced by 500 µg/kg of apomorphine. Animals were pretreated with LHRH (filled circles) or solvent (open circles). Comparisons were made by using Mann-Whitney U-test (*$p<0.05$ and **$p<0.005$). The number of animals in each group was 10.

various doses of apomorphine on conditioned performance are shown in Fig. 5. Kruskal-Wallis ANOVA from all groups indicated significant group differences, $H(9)=57.43$, $p<0.005$. Increased dosages of apomorphine induced a biphasic effect on the acquisition of CARs. In fact, Mann-Whitney U-tests indicate that apomorphine 125 µg/kg significantly impaired acquisition ($p<0.005$) and that apomorphine 500 µg/kg improved CAR performance ($p<0.01$). The effects of apomorphine 62.5 µg/kg and µg/kg were not significant. Pretreatment with LHRH impaired the acquisition of CARs in the controls and in the animals who received apomorphine 62.5 µg/kg, did not modify the effect of apomorphine 125 µg/kg and potentiated the effects of apomorphine 250 µg/kg and 500 µg/kg. It must be noted that the LHRH-induced impairment in the acquisition performance was antagonized by the two higher doses of apomorphine ($p<0.005$).

The acquisition performance across six blocks of ten trials are plotted in Fig. 6. Figure 6A shows that the acquisition performance displayed by the animals injected with 62.5 µg/kg of apomorphine was significantly impaired in blocks 5 and 6. In Fig. 6B, the impairment in the acquisition rate induced by apomorphine 125 µg/kg was partially, but significantly, antagonized by LHRH in blocks 3 and 4. Figure 6C and D show inverted U-shaped effects of apomorphine 250 and 500 µg/kg on the acquisition performance. In fact there is an increase in CARs from block 1 through blocks 3 and 4, respectively, and after that the performance decreased significantly. Consequently, the acquisition of CARs in block 6 was almost completely extinct. This decay in the response was not evident in the animals pretreated with LHRH, whose performance was maintained high at least during the test session.

DISCUSSION

The present results indicate that exogenously administered LHRH is able to modify the behavioral effects induced

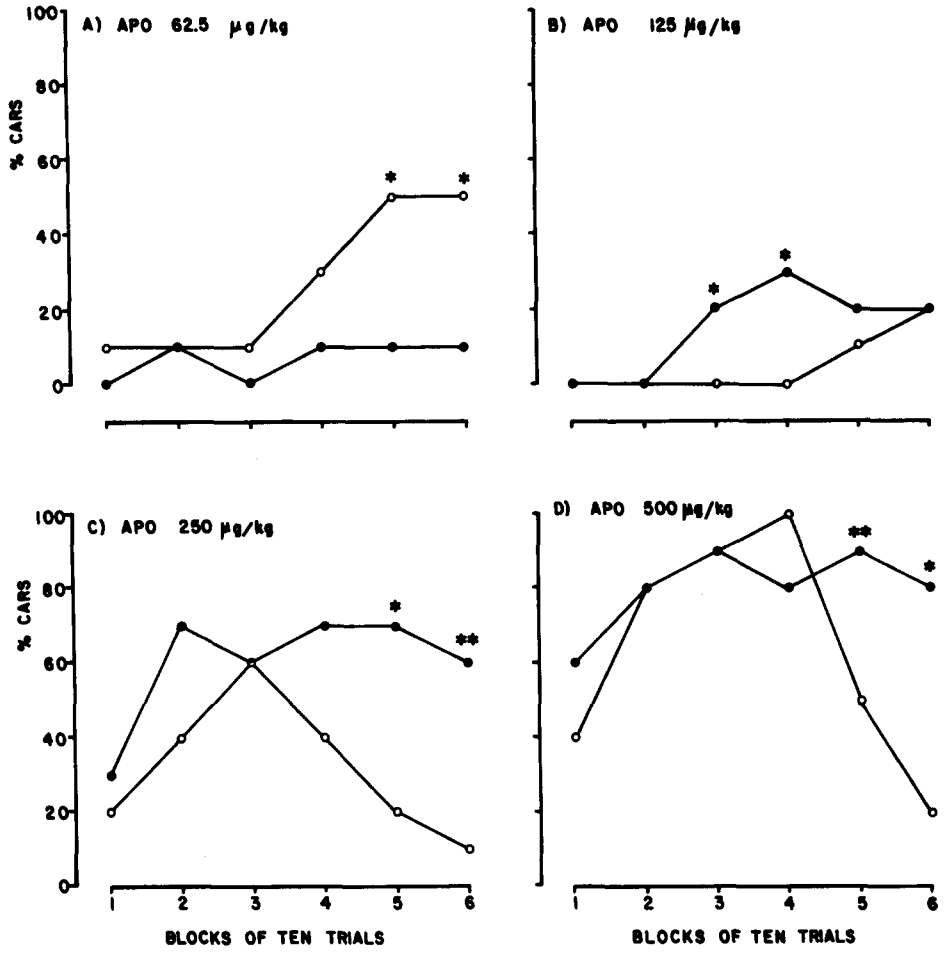

FIG. 6. Influence of the pretreatment with LHRH 100 µg/kg SC and four doses of apomorphine (APO) on the acquisition of conditioned avoidance responses (CARs). Each point of the curve represents the median of the percent of CARs by blocks of 10 successive trials. Mann-Whitney U-test was used to assess differences between specific pairs of medians (*$p<0.05$ and **$p<0.005$). The number of animals in each group was 9.

by apomorphine. The interaction between LHRH and apomorphine was more evident in behavioral responses such as acquisition of CARs, motor activity and stereotyped sniffing. The apomorphine-induced effects on these behaviors were potentiated by the pretreatment with LHRH.

The complex behavioral effects of apomorphine are considered to be due to stimulation of different populations of DA receptors in the CNS. Commonly, the inhibitory effects of low doses of apomorphine on spontaneous or locomotor activity are attributed to stimulation of DA autoreceptors (5). On the other hand, hypermotility and stereotyped behaviors elicited by moderate and high doses of apomorphine are thought to be associated with activation of postsynaptic DA receptors (1,8).

Our study demonstrates that apomorphine exerts a biphasic dose-dependent effect on acquisition of a conditioned response. Apomorphine 125 µg/kg induced an impairment similar to that induced by neuroleptics and higher doses showed an enhancement in the CAR performance. Nevertheless, the latter effect of apomorphine is very short lasting and it is pursued by a disruption of the response.

We have observed that LHRH injected either subcutaneously (10) or intracerebrally in the nucleus caudatus (15) induces an impairment in the acquisition of a conditioned response similar to that produced by low doses of apomorphine, without modifying spontaneous motility. Inhibition of conditioned behavior in animals, without affecting escape responding (18), is an effect generally considered as a characteristic action of almost all drugs which block central dopamine transmission and has been classically used for detection of potential antipsychotic action in man (2). A disruption of striatal DA function is thought to underlie these behavioral changes (9,21).

In our experimental conditions moderately high doses of apomorphine masked the impairing effect of LHRH on the acquisition of CARs. Moreover, the transient improving effects of high doses of apomorphine were potentiated by LHRH treatment. These findings rule out the possibility that the inhibitory effect of LHRH be due to a blockade of postsynaptic DA receptors, but they suggest an increase in the sensitivity of these receptors.

LHRH did not elicit changes in spontaneous motility by

itself, but potentiated both hypomotility and hypermotility induced by low and high doses of apomorphine, respectively. In addition, stereotyped sniffing produced by 500 μg/kg of apomorphine was also potentiated by LHRH. Other investigators have shown that LHRH pretreatment can potentiate the effects of dopaminergic drugs, demonstrating that large doses of LHRH among other hypothalamic hormones potentiated the behavioral effects of L-DOPA in mice (19).

Taken together, the findings related above led to the suggestion that LHRH indirectly regulates DA activity. Since the behavioral patterns induced by DA agonists have been linked with the function of the striatum (3,6), we postulate that LHRH might also influence striatal DA activity. Although the mechanism is unclear, the potentiation of apomorphine-induced effects could be the consequence of an increase in the sensitivity of DA receptors in the striatum. It is known that the responsiveness of a drug can be altered by different mechanisms, e.g., denervation or chronic treatment with drugs influencing synaptic transmission. Therefore receptor hypersensitivity can be developed following understimulation.

There is behavioral and biochemical evidence supporting the hypothesis of a presynaptic inhibitory effect of LHRH upon synthesis and release of DA. Recent reports indicate that LHRH, in addition to its inhibitory effect on conditioning, antagonizes the amphetamine-induced effects on conditioning and motor activity (11). L-DOPA, the precursor of catecholamine synthesis, antagonizes the LHRH-induced impairment on conditioning and counteracts the antagonism between LHRH and amphetamine on conditioning and motor activity (14). It has been demonstrated that the incubation of corpus striatum synaptosomes in the presence of LHRH (5×10^{-6} M) has inhibitory effects on DA synthesis (23). A study performed in our laboratory showed that LHRH 100 μg/kg, subcutaneously injected, is able to decrease synthesis and release of DA from rat corpus striatum slices, thus correlating the behavioral effects of LHRH with biochemical changes in striatal DA transmission (16).

In conclusion, the present study contributes to further support the hypothesis of a modulatory-like action of LHRH on DA systems. It is shown that the acute treatment with a single dose of LHRH potentiates the behavioral pattern induced by apomorphine. This effect could be attributed to an increase in DA receptors sensitivity, secondary to an impairment in presynaptic DA activity.

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